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Summary of cosmid clones isolated from *M. echinospora* genomic library.

clone ^a	type I PKS genes ^b	type II PKS genes ^b	deoxy sugar genes ^b	resistance ($\mu\text{g mL}^{-1}$) ^c
3a	N.D. ^d	N.D. ^d	N.D. ^d	0.5
4a	N.D. ^d	N.D. ^d	N.D. ^d	0.5
4b	+	+	+	0.5
10a	+	+	+	0.5
13a	+	+	+	0.5
16a	N.D. ^d	N.D. ^d	N.D. ^d	0.5
56	+	+	+	0.1
58	-	-	+	< 0.01
60	+	+	+	0.05
66	-	-	+	0.04
puc18/pBluescript ^e	-	-	-	< 0.01

Fig. 1



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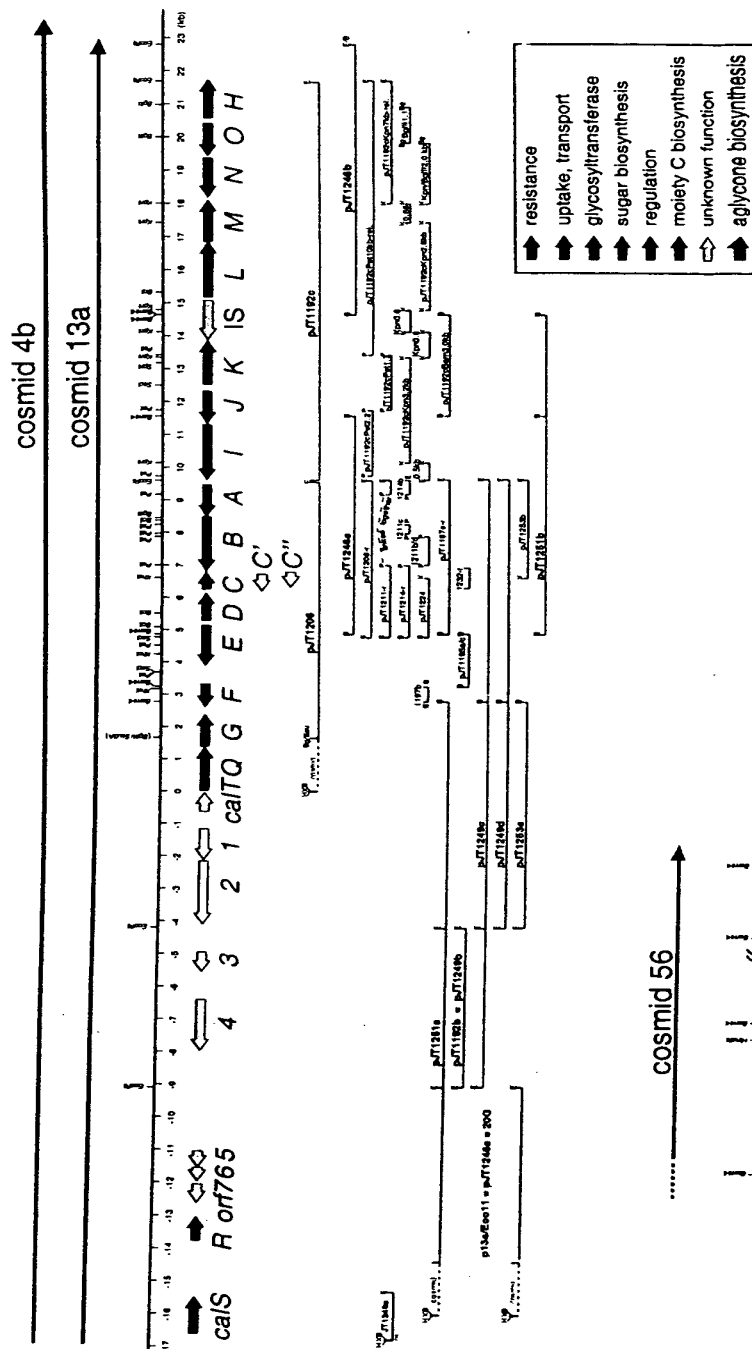
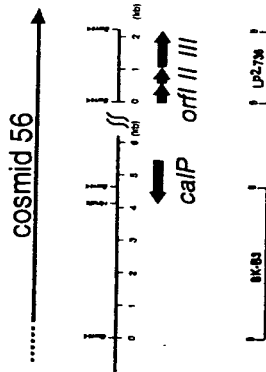


Fig. 2





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putative polypeptide	number of amino acids	proposed function or sequence similarity detected ^a	probability ^b	start/stop codons	best match
CalA	328	membrane transporter (ATP-binding)	5.4×10^{-124}	ATG/TGA	DrrA ⁹⁷
CalB	561	membrane transporter	5.5×10^{-70}	ATG/TGA	DrrB ⁹⁷
CalC	181	calicheamicin resistance protein	confirmed ^c	ATG/TGA	
CalD	263	O-methyltransferase	1.1×10^{-99}	ATG/TGA	AveBVII ⁹⁸
CalE	420	Glycosyltransferase	4.7×10^{-30}	GTG/TAG	EryCII ⁹⁹
CalF	245	N,N-dimethyltransferase	1.5×10^{-78}	ATG/TGA	DesVI ¹⁰⁰
CalG	990	TDP-D-glucose 4,6-dehydratase	confirmed ^c	GTG/TAG	
CalH	338	Perosamine synthetase	confirmed ^c	GTG/TGA	
CalI	568	Dipeptide transporter	1.7×10^{-24}	GTG/TGA	DciAE
CalJ	332	O-methyltransferase	1.0×10^{-37}	ATG/TGA	DmpM
CalK	440	L-cysteine/cystine C-S-lyase	1.6×10^{-28}	GTG/TGA	C-DES
CalL	562	Oligopeptide transporter protein	9.5×10^{-14}	ATG/TGA	OppA
CalM	416	Regulatory protein		GTG/TGA	
CalN	398	Glycosyltransferase	3.4×10^{-79}	ATG/TGA	OleI
CalO	331	Hexopyranosyl-2,3-reductase	4.9×10^{-139}	ATG/TGA	EryBII
CalP	(179) ^d	Desaturase	5.7×10^{-7}	/TGA	CrtI
CalQ	453	UDP-D-glucose 6-dehydrogenase	confirmed ^c	GTG/TGA	
CalR	282	Transcriptional regulator	6.7×10^{-11}	ATG/TGA	SC5C7.03
CalS	1113	P ₄₅₀ oxidase	2.9×10^{-66}	GTG/TGA	Biol
CalT	432	oxygenase/halogenase	2.0×10^{-62}	GTG/TAA	PCZA361.21
CalU	377	glycosyltransferase	2.0×10^{-53}	ATG/TGA	SnogE/D
CalV	125	β-keto-acyl synthase III	2.0×10^{-65}	ATG/TGA	SC4A9
CalW	(449) ^d	cytochrome P450	1.0×10^{-91}	GTG/TGA	CYP105B1
CalX	(197) ^d	TDP-4-keto-6-deoxy-L-hexose 2,3-dehydratase	1.0×10^{-22}	/TGA	MtmV
6MSAS	(198) ^d	orsellenic acid synthase	6.5×10^{-76}	ATG/	AviM
ActI	(207) ^d	polyketide cyclase	3.0×10^{-66}	/TGA	CurF
ActII	136	polyketide cyclase	5.0×10^{-53}	ATG/TGA	SchB
ActIII	(308) ^d	polyketide synthase	8.6×10^{-148}	GTG/	Pms1
orf1	322	unknown		ATG/TGA	
orf2	654	unknown		ATG/TGA	
orf3	373	integrase	3.0×10^{-13}	ATG/TGA	Yld
orf4	521	chromosome partitioning protein	3.3×10^{-10}	GTG/TAA	ParA
orf5	175	unknown		ATG/TGA	
orf6	139	unknown		ATG/TGA	
orf7	187	unknown		GTG/TGA	
orf8	266	regulatory protein	3.0×10^{-66}	ATG/TGA	KorSA
OrfI	127	hydroxylase	1.5×10^{-7}	ATG/TGA	SC4C6.24c
OrfII	248	unknown		GTG/TGA	
OrfIII	298	hydroxylase	3.3×10^{-90}	GTG/TGA	SCA32
OrfIV	363	unknown	5.3×10^{-43}	GTG/TGA	SC9C7.25
OrfV	288	aminotransferase	2.9×10^{-37}	GTG/TGA	SCF55
OrfVI	1012	glu-ammonia-ligase adenyltransferase	exact	GTG/TGA	SCA32
OrfVII	236	Methyltransferase	8.0×10^{-63}	GTG/TAG	SCF43A.25c
OrfVIII	441	Integral membrane protein	8.9×10^{-9}	GTG/TGA	SCA32
OrfIX	478	Integral membrane protein	1.1×10^{-21}	ATG/TGA	MLB268
OrfX	504	Membrane protein	5.5×10^{-20}	GTG/TGA	B1496.F1.14
OrfXI	251	Immunity resistance protein	1.1×10^{-9}	ATG/TGA	TFXG
IS-element	1209 bp	insertional element	5.7×10^{-108}		IS1136 ¹¹¹

Fig. 3.



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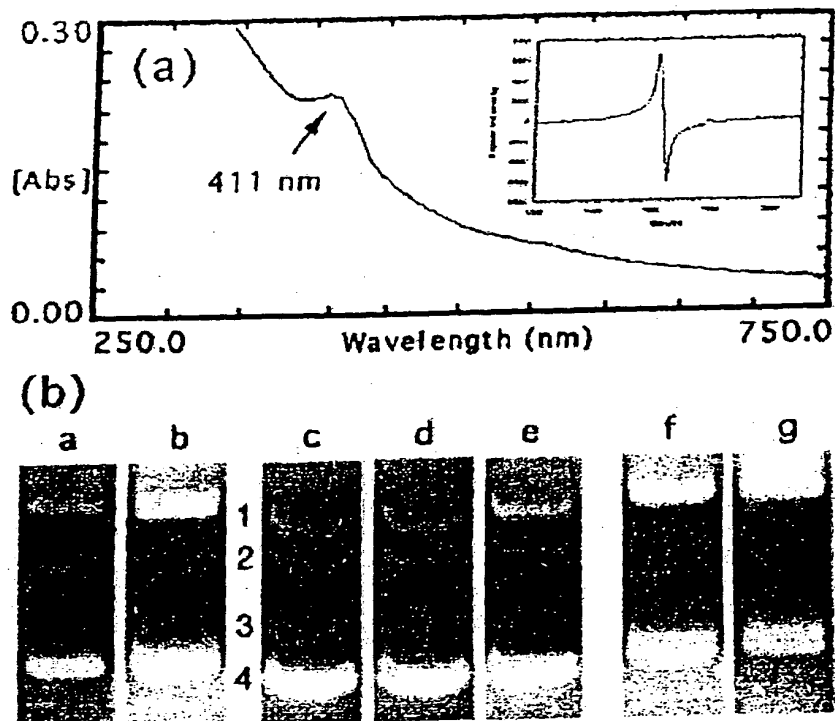


Fig. 4

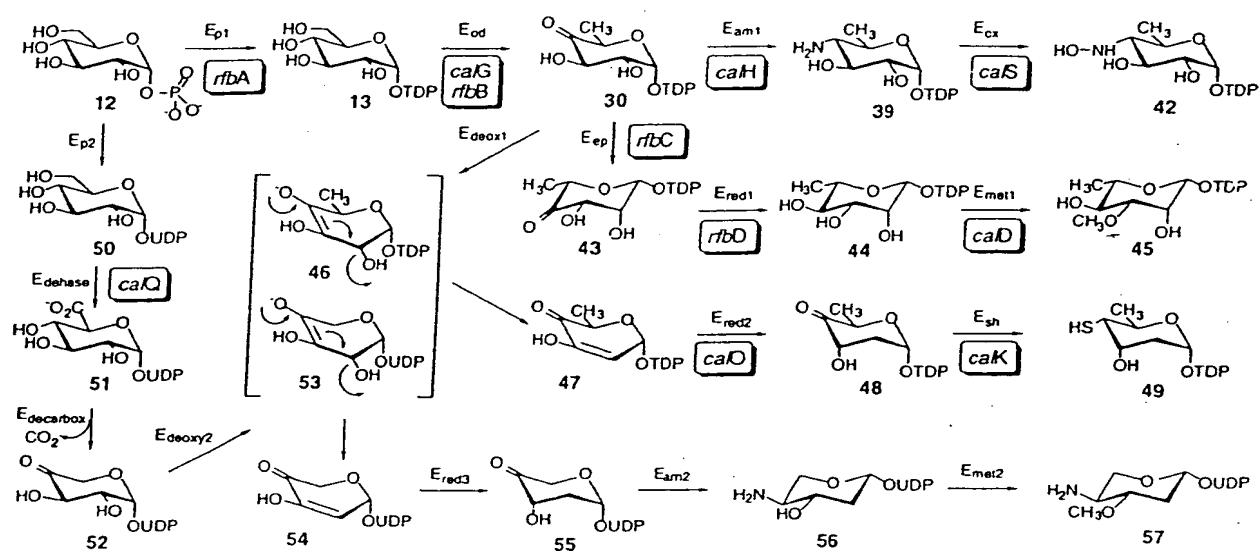


Fig. 5

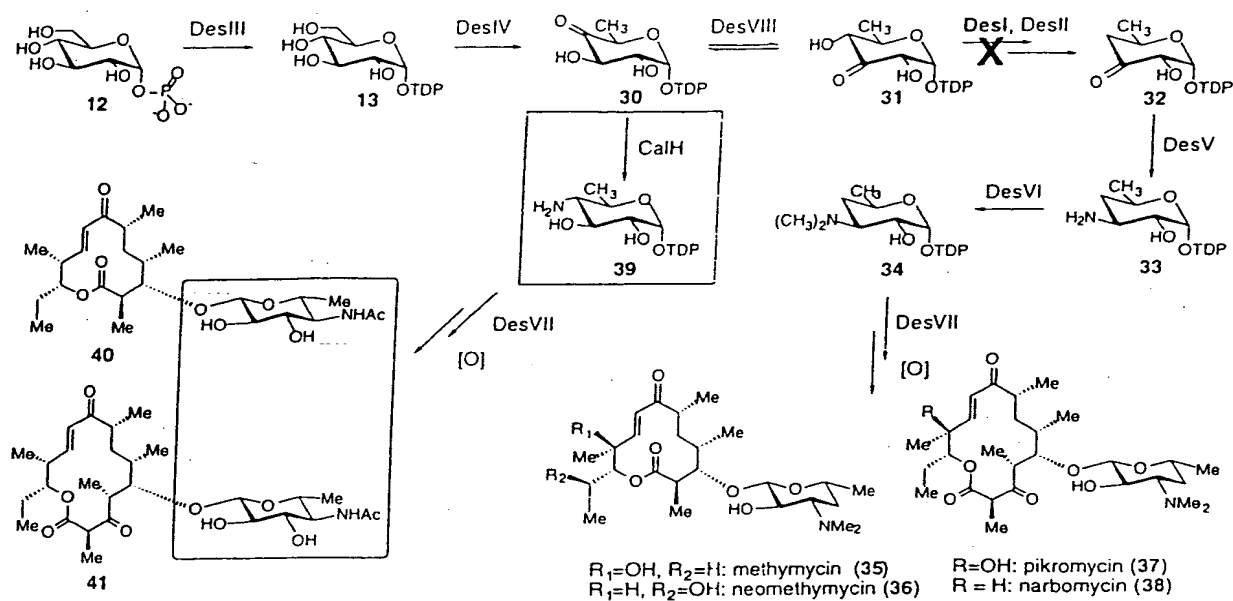


Fig. 6

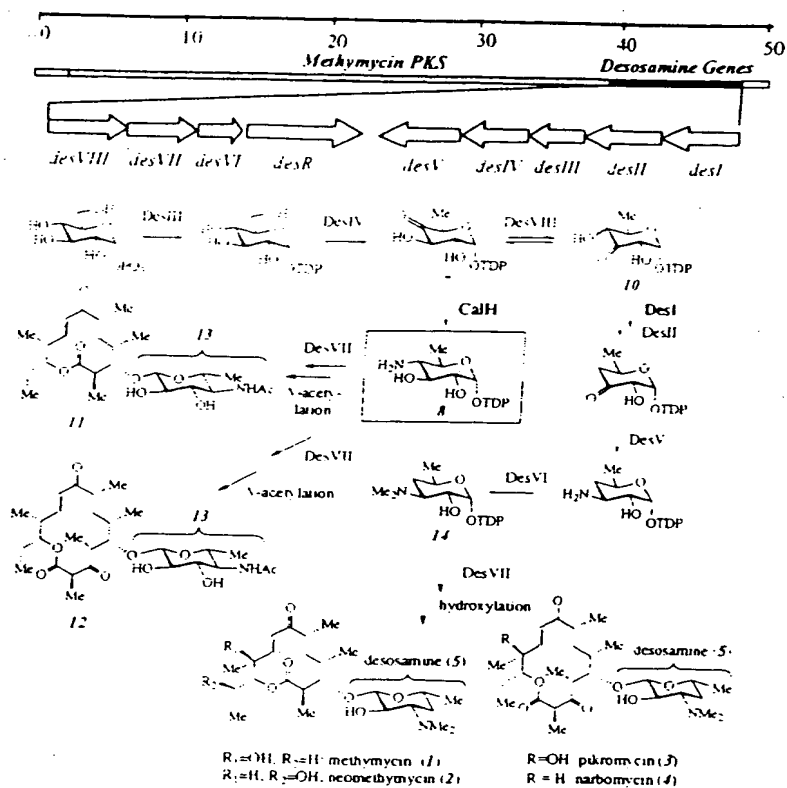


Fig. 7

Fig. 8

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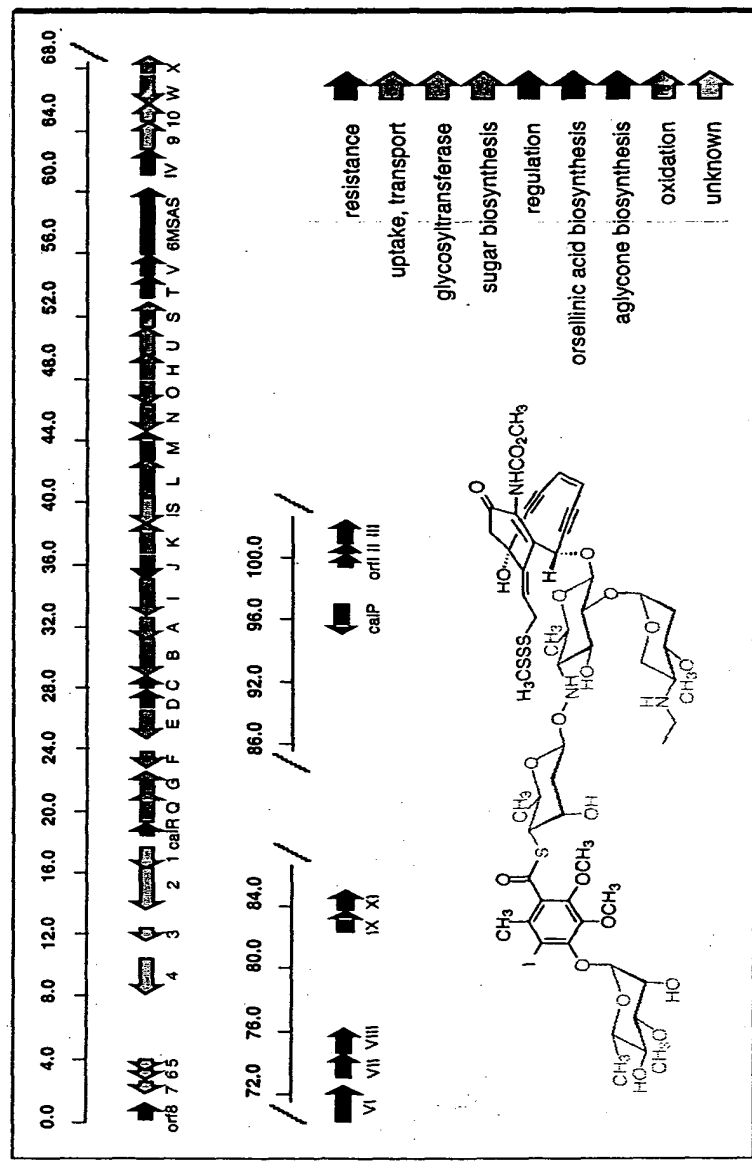
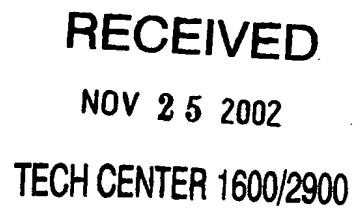


Fig 9

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Type I PKS
de novo polyketide
biosynthesis

6-MSAS $\xrightarrow{\text{calV}}$

"halogenase" $\xrightarrow{\text{calT}}$

oxidation $\xrightarrow{\text{calS calW}}$

SAM-dependent methylation $\xrightarrow{\text{calD calJ}}$

FINAL ASSEMBLY

The diagram illustrates the final assembly steps of Type I PKS de novo polyketide biosynthesis. It begins with 6-MSAS, which is converted to a hydroxy-ketone intermediate by the enzyme CalV. This intermediate then undergoes three parallel modifications: halogenation by CalT, oxidation by CalS and CalW, and SAM-dependent methylation by CalD and CalJ. The final product is a complex polyketide chain with multiple functional groups.

Synthesis of Putative Substrates:

The reaction scheme illustrates the synthesis of two putative substrates from a common intermediate, 4-methoxy-3-methylbenzoic acid.

Starting Material: 4-methoxy-3-methylbenzoic acid

Reaction 1: 4-methoxy-3-methylbenzoic acid reacts with CuBr_2 to form 4-methoxy-3-methylbenzyl bromide.

Reaction 2: 4-methoxy-3-methylbenzoic acid reacts with $n\text{-BuLi}$ and CO_2 to form 4-methoxy-3-methylbenzoic acid.

Reaction 3: 4-methoxy-3-methylbenzoic acid reacts with 1) AlCl_3 and 2) NaOH to form 3-methyl-4-(carboxymethyl)benzoic acid.

Reaction 4: 4-methoxy-3-methylbenzoic acid reacts with NaOH to form 3-methyl-4-(methoxycarbonyl)benzoic acid.

Fig 11

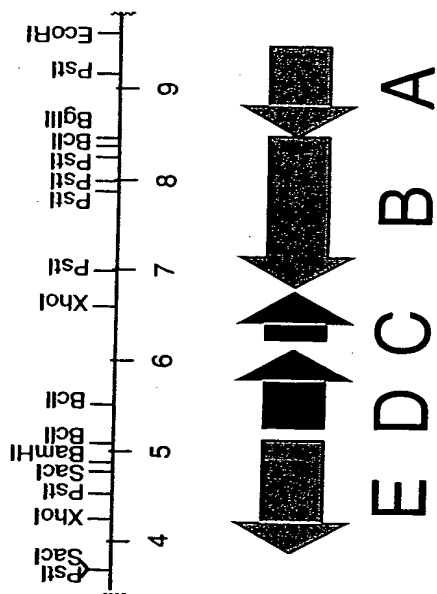
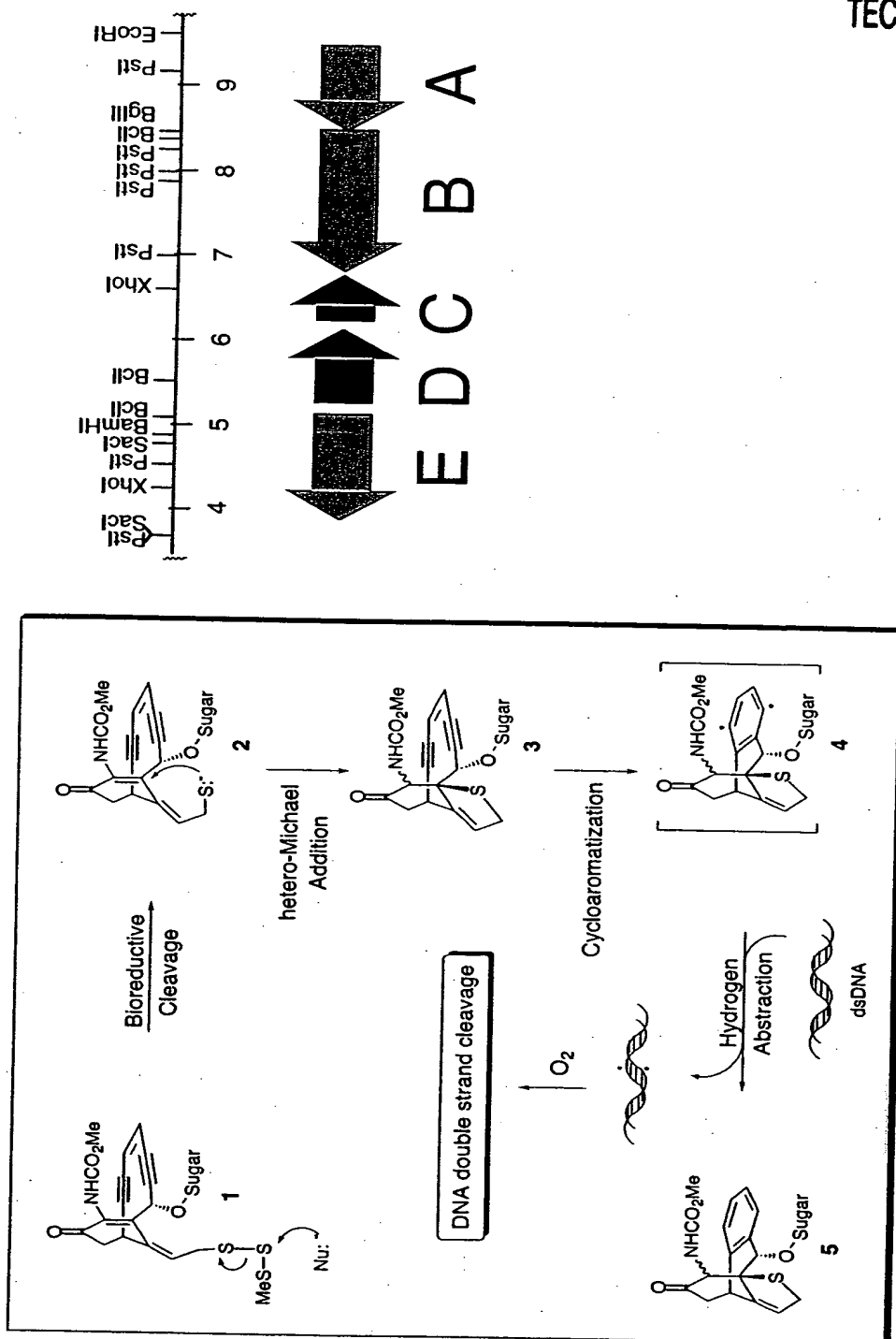


Fig 12

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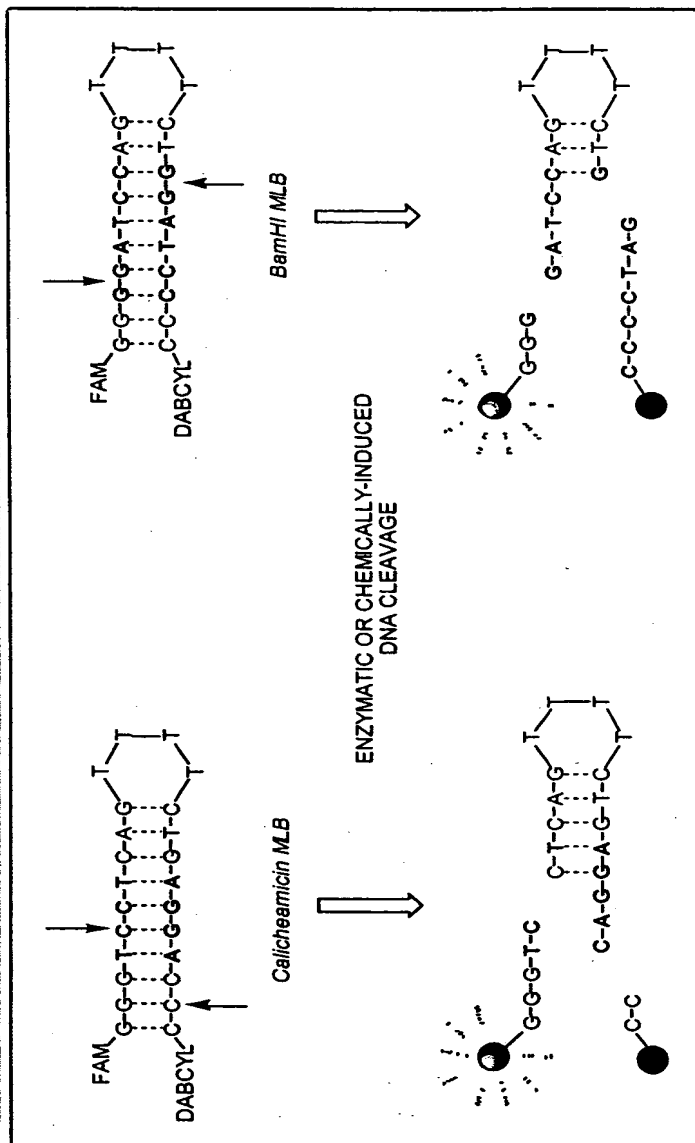


Fig 13



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1 U DNaseI

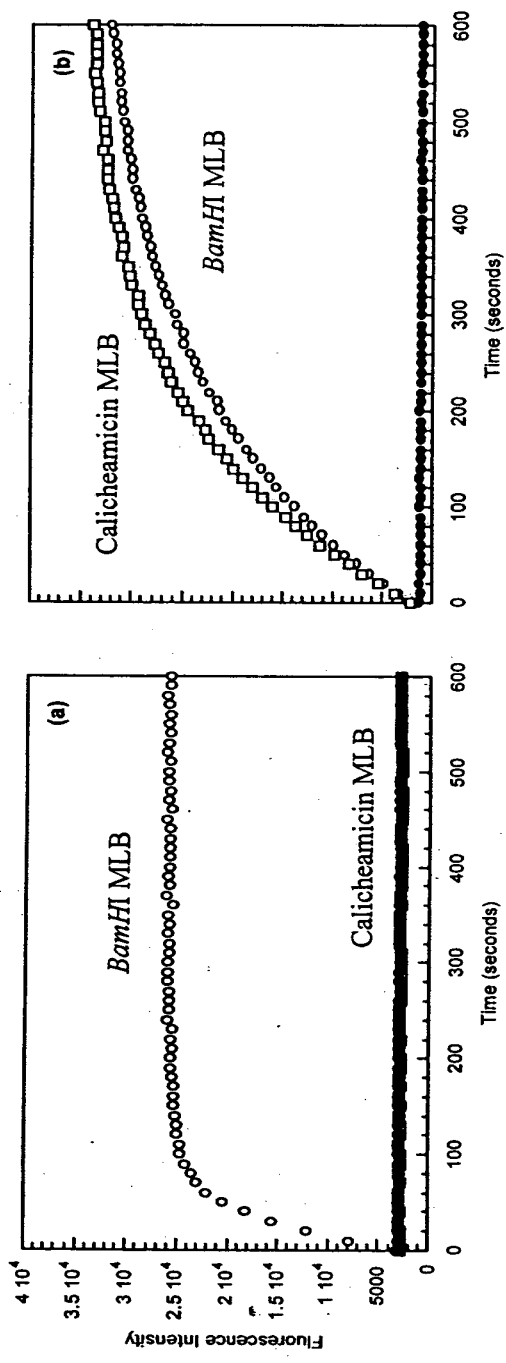


Fig 14



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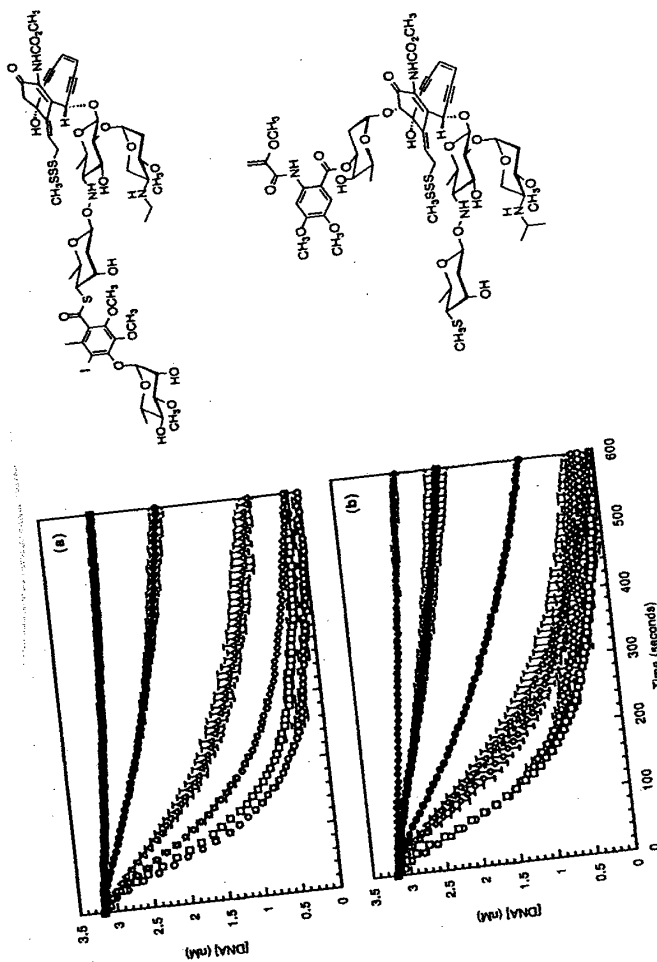
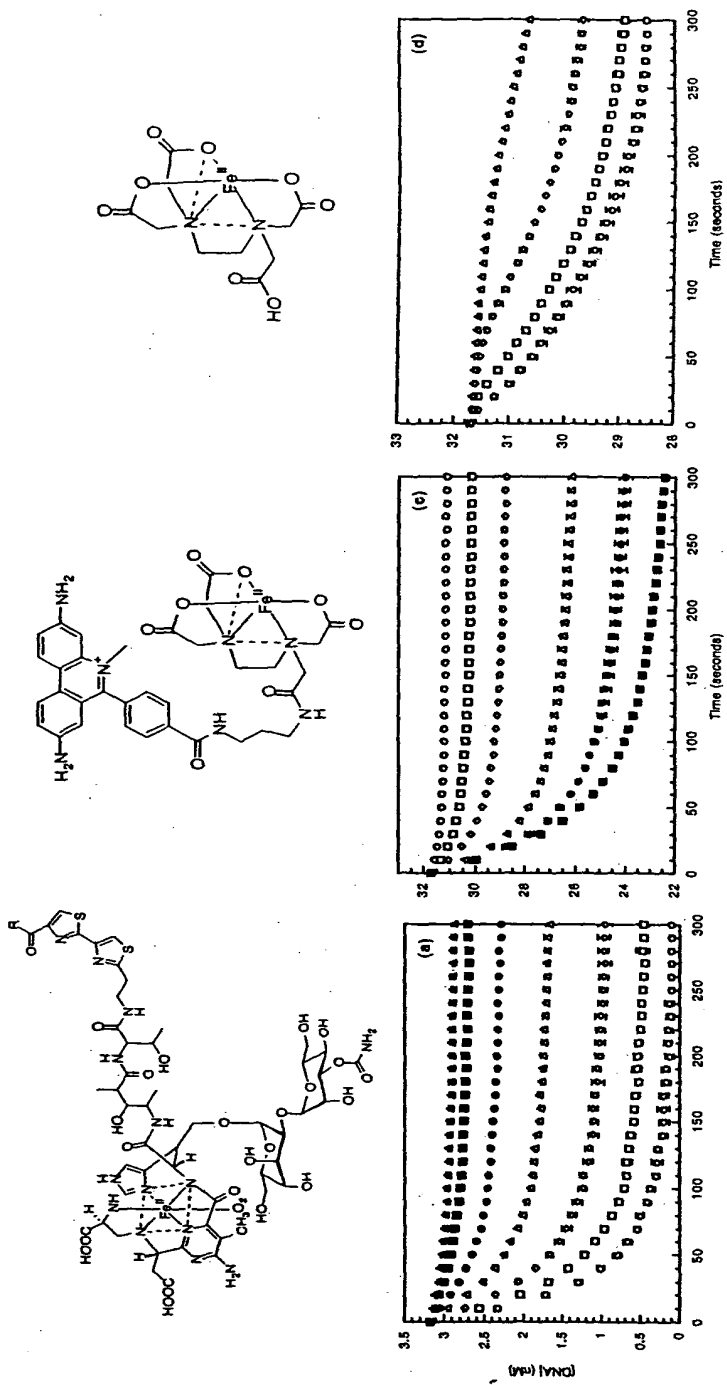


Fig 15



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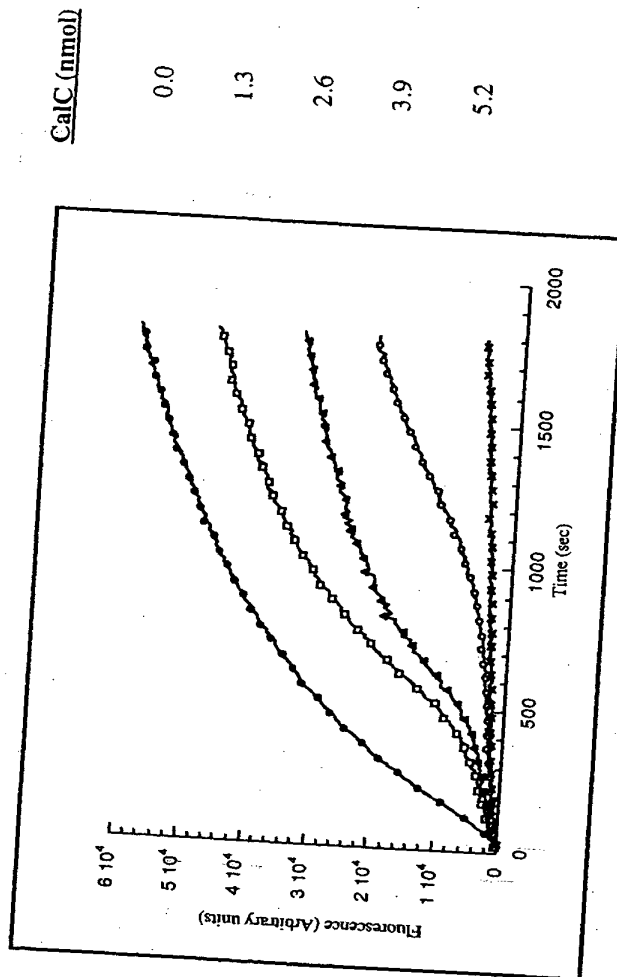


Fig 17

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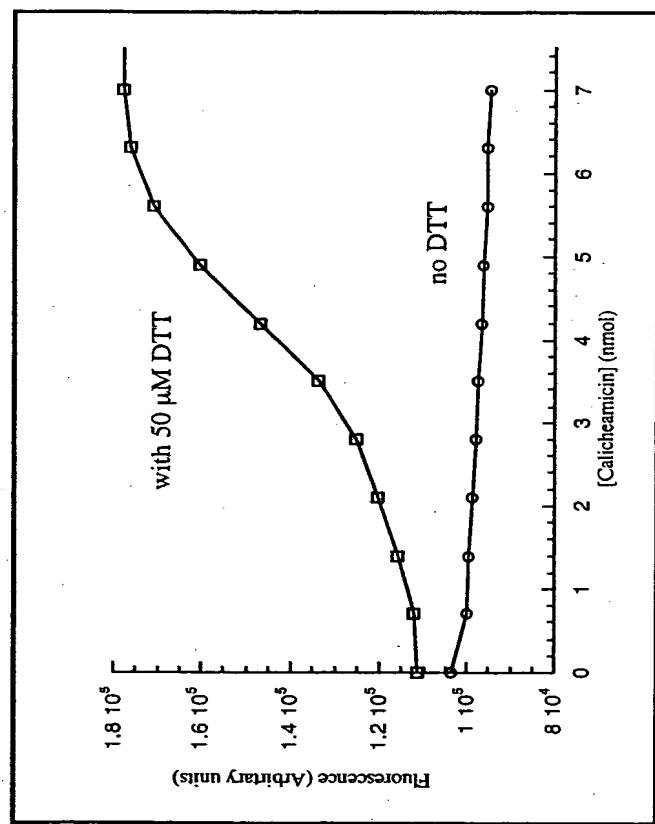
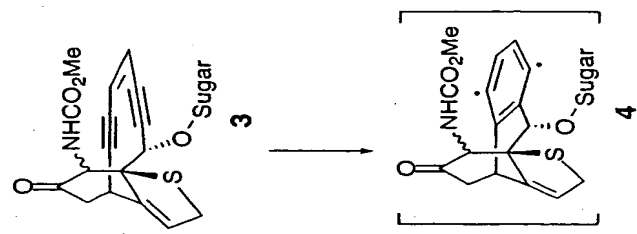


Fig 18